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TRISODIUM NITRILOTRIACETATE AND ALGAE

DIVISION OF RESEARCH

ONTARIO WATER RESOURCES COMMISSION

June, 1969

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TRISODIUM NITRILOTRIACETATE AND ALGAE

By:

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June, 1969

Division of Research
Paper No. 2023

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SUMMARY

The relationships between trisodium nitrilotriacetate, a potential detergent builder, and the alga *Chlorella pyrenoidosa* were investigated with respect to toxicity and as a source of nitrogen both before and after treatment of the material in an acclimatized activated sludge system. The compound is non-toxic to *Chlorella* at a concentration of 275 mg/l and can act as a source of nitrogen for algal growth when fed directly or after treatment in an activated sludge system.

INTRODUCTION

Trisodium nitrilotriacetate ($NTANa_3$) is one of several compounds being investigated by detergent and chemical manufacturers seeking a replacement for phosphate builders used in detergents (3).

$NTANa_3$ is non-toxic to fish at concentrations up to 100 mg/l, to mammals fed up to several thousand milligrams per kilogram body weight, and non-irritating to human skin (4). The chemical also appears to be biodegraded quite readily by activated sludge after an acclimatization period of from one to two weeks (1, 2, 6) and does not affect sludge synthesis, sludge settling or inhibit the breakdown of LAS, phenols or domestic sewage components.

As as amino carboxylic acid, NTA obviously contains nitrogen, which like phosphorus, is essential for the growth of algae. Relationships between $NTANa_3$ and the alga Chlorella pyrenoidosa with respect to toxicity and as a nitrogen source were examined in the following investigation.

METHODS AND MATERIALS

Axenic cultures of the alga *Chlorella pyrenoidosa* Chick (#395 Indiana Culture Collection) (5) were grown in the following media (mg/l): $NaNO_3$ - 250, $CaCl_2$ - 25, $MgSO_4 \cdot 7H_2O$ - 75, KH_2PO_4 - 175, NaCl - 25, for at least seven days at 20°C at 4000 footcandles in a 14:10 hour light/dark cycle.

Cultures for testing the availability of $NTANa_3$ (J. T. Baker Chemical Company) as a nitrogen source consisted of 50 ml of the mineral media, minus $NaNO_3$, contained in 170 ml sterilized bottles mounted on a shaker table, to which 0.1 ml of material from an axenic culture of *Chlorella* was added. Enumeration of samples was carried out by counting the number of viable cells in six fields using a haemacytometer and expressing the results as cells/ml.

An activated sludge capable of degrading $NTANa_3$ was developed by placing 5 litres of mixed liquor solids, about 3000 mg/l, in a plastic tank which over a 6 week period was fed increasing quantities of $NTANa_3$, up to 275 mg/l. The original mixed liquor solids were obtained from an activated sludge stock system which had only been fed domestic sewage. Twenty-four hours after the last feeding of $NTANa_3$ the sludge system was washed six times during a 12 hour period with distilled water. After washing, 100 ml aliquots of the material were placed in sterile 170 ml bottles containing $NTANa_3$. These samples were then aerated for six hours, allowed to settle for 30 minutes and the supernatant filtered through 0.45 micron membranes. A 50 ml aliquot of the filtrate was then placed in a sterile 170 ml bottle and inoculated with 0.1 ml of material from an axenic *Chlorella* culture. A 0.5 ml aliquot of a KH_2PO_4 solution equivalent to 175 mg/l was also added.

All experiments were replicated three times.

EXPERIMENTATION

Preliminary experiments to investigate the role of $NTANa_3$ as a source of nitrogen for algae were carried out by measuring the growth of *Chlorella pyrenoidosa* in a mineral media (approximately pH 6.9) to which equivalent quantities of nitrogen were added either as $NTANa_3$ or $NaNO_3$ without (Table IA) or with (Table IB) the addition of a small quantity of raw sewage, the latter in an attempt to approximate a raw water situation.

Preliminary testing of $NTANa_3$ toxicity to algae was accomplished by adding the compound to week old cultures of *Chlorella* grown in a complete mineral media. Responses of the treated cultures after an exposure of 7 days are shown in Table IC.

$NTANa_3$ as a nitrogen source for algae was further investigated over a concentration range of 0.014 - 14.0 mg/l N (0.275 - 275.0 mg/l $NTANa_3$) using an otherwise nitrogen free mineral media and noting the response of *Chlorella* after a period of 14 days (Table II).

The relative availability of $NTANa_3$ and its by-products as a nitrogen source for algae after treatment of the substance in an acclimatized activated sludge system was observed by culturing Chlorella in the filtered supernatants of aliquots of the activated sludge which had received various concentrations of $NTANa_3$. Characteristics of the activated sludge system after washing, prior to experimentation, are listed in Table III. Responses of Chlorella in the various supernatants after a period of 14 days are shown in Table IV.

TABLE I

Results of preliminary experiments with $NTANa_3$ (202.3 mg/l) and $NaNO_3$ (62.5 mg/l) and responses of Chlorella pyrenoidosa after an exposure of 7 days

	10.3 mg N/l	10^6 cells/ml
A)	as a N source - bacteria absent	
	$NTANa_3$	0.55
	$NaNO_3$	1.20
B)	as a N source - bacteria present	
	$NTANa_3$	0.35
	$NaNO_3$	1.06
C)	toxicity to 7 day axenic cultures of algae	
	$+NTANa_3$	4.98
	$-NTANa_3$	3.46

TABLE II

Growth of Chlorella pyrenoidosa after 14 days in a mineral media in which $NTANa_3$ or $NaNO_3$ acted as the source of nitrogen

	10^6 cells/ml	
mg N/1	$NTANa_3$	$NaNO_3$
0.014	1.84	2.70
0.14	1.91	2.76
1.4	3.67	3.99
14.	4.27	7.52

TABLE III

Characteristics of the acclimatized activated sludge system before experimentation with $NTANa_3$

Supernatant	mg/l
COD	5
TOC	6
$N:NO_3$	1.80
$N:NO_2$	0.05
$N:NH_3$	0.28
N:Kjeldahl	0.80
P:total	0.42
Mixed Liquor	
Suspended Solids	2960
Volatile Suspended Solids	1950

TABLE IV

Growth of Chlorella pyrenoidosa after 14 days when cultured in filtered supernatants of activated sludge treated with $NTANa_3$

mg N/1 added	10^6 cells/ml
0.0	0.88
0.014	1.72
0.14	1.30
1.4	0.93
14.	9.10

DISCUSSION AND RECOMMENDATION

The results of these experiments indicate that $NTANa_3$ at concentrations up to 275 mg/l is not toxic to the test organism Chlorella pyrenoidosa and can act as a source of nitrogen for algal growth (Table I, II), albeit not to the same extent as equivalent quantities of nitrogen fed as nitrate under the same test conditions.

Algal responses in supernatants of acclimatized activated sludge treated with various concentrations of $NTANa_3$ are listed in Table IV. When these data were analyzed statistically, no significant difference was found to exist between the control and all responses except those related to the highest concentration of $NTANa_3$ fed. Examination of the characteristics of the sludge supernatant (Table III) suggest that this apparent lack of significant difference in the response of Chlorella to $NTANa_3$ treatments of 27.5 mg/l or less probably results from a masking of the $NTANa_3$ addition by the nitrogen content of the sludge supernatant. These results do show however that treatment of 275 mg/l $NTANa_3$ by an acclimatized activated sludge did not reduce the algal growth potential of the chemical.

As carbon rather than nitrogen or phosphorus is the nutrient usually limiting bacterial action in sewage treatment plants, it is suggested that the feasibility of biodegradable organic materials as detergent builders which contain neither nitrogen or phosphorus should be thoroughly considered.

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